UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

January 20, 2010

MEMORANDUM

Subject: Efficacy Review for AdvaCare 120 Sanitizer/Sour; EPA Reg. No. 1677-193; DP Barcode: D371317

From: Ibrahim Laniyan, Microbiologist
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1/22/10

To: Marshall Swindell / Martha Terry
Regulatory Management Branch I
Antimicrobials Division (7510P)

Applicant: Ecolab Inc.
370 N. Wabasha Street
St. Paul, MN 55102

Formulation from the Label:

Active Ingredient	% by wt.
Peroxyacetic acid	15.2 %
Hydrogen peroxide	11.2 %
Inert Ingredients	73.6 %
Total	100.0 %

I. BACKGROUND

The product, AdvaCare 120 Sanitizer/Sour (EPA Reg. No. 1677-193), is an EPA-approved laundry sanitizer for use in commercial, institutional, and industrial laundry operations. The applicant requested to amend the registration of this product to add claims for effectiveness as a laundry disinfectant. The label states that the product is effective in water up to 500 ppm of water hardness. Studies were conducted at Ecolab Research Center – Ecolab Schuman Campus, located at 655 Lone Oak Drive in Eagan, MN 55121.

This data package contained a letter from the applicant to EPA (dated October 23, 2009), two studies (MRID 478975-01 and 478975-02), Statements of No Data Confidentiality Claims for both studies, and the proposed label (dated October 15, 2009).

II. USE DIRECTIONS

The product is designed for use as a laundry disinfectant during commercial-industrial-institutional operations. Directions on the proposed label provide the following information regarding use of the product: Inject the product into the bleach or rinse step. Use at a rate of 4 ounces of the product per maximum 60 gallons of water (a 1:1919 dilution) to sanitize a maximum of 100 pounds of dry laundry. Treat the laundry for a minimum of 5 minutes at 140-160°F (60-71°C).

III. AGENCY STANDARDS FOR PROPOSED CLAIMS

Laundry Disinfectants: The effectiveness of laundry disinfectants must be supported by data that show that the product will completely kill test bacteria on fabric and in laundry water. Laundry additives may either be used as soaking treatments prior to laundering or as treatments added during laundry operations. The label must specify the type of use. Laundry additives may be recommended for household/coin-operated machine use or commercial-industrial-institutional use. The label must specify the type of use. There is a significant difference in the water to fabric ratio between these two uses, which may affect the efficacy of the product. Tests should be conducted using a simulated-use procedure such as Petrocci and Clarke's "Proposed Test Method for Antimicrobial Laundry Additives" or a simulated use study involving washing machines. Tests should be performed with each of 3 product samples, representing 3 different product lots, one of which is at least 60 days old. Tests should be conducted against *Staphylococcus aureus* (ATCC 6538) and *Klebsiella pneumoniae* (ATCC 4352). Products labeled as being suitable for hospital use must also be tested against *Pseudomonas aeruginosa* (ATCC 15442). Each product lot must be tested with 9 fabrics swatches against each of the test organisms. The method employed must include subculturing of both the fabric and the laundry water. The laundry water to media volume ratio must not exceed 1:40. Testing of a 0.5 mL sample of laundry water from the simulated washing device (or a 5 mL sample from the automatic washer) is recommended. Results must be reported after a 48-hour incubation period. Results must show no growth in the fabric subcultures and no growth in the laundry water subcultures. The label directions for use of laundry additives should specify the machine cycle in which the product is to be added, as well as water level, temperature, and treatment time. Compatibility of the treatment with other laundry additives should be determined in testing and addressed in labeling, when applicable. These Agency standards are presented in

DIS/TSS-13, and do not apply to sodium-calcium hypochlorites, sodium-potassium dichloro-s-triazinetriones, or trichloro-s-triazinetrione.

Note: The water to fabric ratio in industrial laundering operations is about 5:1. Dosages may be based on pounds of fabric for industrial machines.

IV. COMMENTS ON THE SUBMITTED EFFICACY STUDIES

1. MRID 478975-01 "AdvaCare 120 Laundry Additive Disinfection Efficacy," Test Organisms: *Klebsiella pneumoniae* (ATCC 4352), *Staphylococcus aureus* (ATCC 6538), and *Pseudomonas aeruginosa* (ATCC 15442), by Laurinda Holen. Study conducted at Ecolab Research Center – Ecolab Schuman Campus. Study completion date – October 5, 2009. Study Identification Number 0900023.

This study was conducted against *Klebsiella pneumoniae* (ATCC 4352), *Staphylococcus aureus* (ATCC 6538), and *Pseudomonas aeruginosa* (ATCC 15442). Three lots (Lot Nos. J031891, J051291, and 060991) of the product, AdvaCare 120, were tested using Ecolab SOP Method MS042-14: "Antimicrobial Laundry Additives" (copy provided). The laboratory report referenced Petrocci and Clarke's "Proposed Test Method for Antimicrobial Laundry Additives." Each of the product lots tested was at least 60 days old at the time of testing. Use solutions of the product were prepared by adding ~1.2 g of the product and 2998.8 mL of 500 ppm AOAC synthetic hard water (titrated at 490-510 ppm; use solution at or below 61.66 ppm peroxyacetic acid/ 44.04 ppm hydrogen peroxide; a 1:2500 dilution). Cultures of the challenge microorganisms were prepared in accordance with Ecolab SOP Method MS042-14 (which is similar to culture preparation described in Petrocci and Clarke's method). Numerous sets of dry, sterile jars were filled with 75 mL of the prepared use solution and equilibrated to 140±2°F. The carriers for this test were prepared by boiling poly-cotton blend or 100% cotton cloth (thread count not specified) in a solution of 300.00 grams of sodium carbonate, 1.50 grams of Triton X-100, and 3 L of Milli-Q water. The fabric then was rinsed in boiling water and then rinsed in cold water. After drying for a minimum of 24 hours at ambient temperature, the fabric was cut into 2 inch wide strips weighing 15±1 grams. Each fabric strip was wrapped around a spindle. Swatches (1 inch by 1.5 inch) were also cut from the remaining fabric. Fabric carriers were sterilized by autoclave. Nine swatches per product lot per test organism were inoculated with 0.02 mL of the prepared organism culture, and dried until visibly dry but for no longer than 30 minutes at 35±2°C. After drying, the swatches were each inserted between the 6th and 7th lap of a wrapped spindle. Each spindle contained three dried, contaminated swatches. Three spindles were prepared for each test organism. The spindles were placed in the dry, sterile jars containing the use solution and subjected to a simulated tumble-wash at 35-60 RPM for 5 minutes at 140±2°F. Within 1 minute, the fabric swatches were transferred to 10 mL of DE Broth to neutralize. The fabric swatches were then vortex mixed well to extract fabric-bound microorganisms. Serial dilutions were prepared, and unspecified dilutions were plated on tryptone glucose extract agar. Also within 1 minute, 25 mL of 2% sodium thiosulfate was added to the wash water after the excess wash water was squeezed from the spindle using a sterile glove. The neutralized wash water was swirled, and a 1 mL aliquot was plated on tryptone glucose extract agar. All subcultures were incubated for 48±4 hours at 35±2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for initial inoculum count, ambient wash water control count, test temperature wash water control count, ambient numbers count, test temperature numbers count, purity, sterility, and neutralization confirmation.

Note: Adding a ca. 15-gram cloth strip to 75 mL of product use solution yields a 1:5 w/v ratio of simulated laundry to wash water, the appropriate ratio for institutional laundry additive testing.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

2. MRID 478975-02 "AdvaCare 120 Supplemental Laundry Additive Disinfection Efficacy," Test Organisms: *Salmonella enterica* (ATCC 10708), *Acinetobacter baumannii* (ATCC 19606), *Staphylococcus aureus* – MRSA (ATCC 33592), *Escherichia coli* O157:H7 (ATCC 43895), *Listeria monocytogenes* (ATCC 49594), and *Enterococcus faecium* VRE (ATCC 51559), by Laurinda Holen. Study conducted at Ecolab Research Center – Ecolab Schuman Campus. Study completion date – October 5, 2009. Study Identification Number 0900024.

This study was conducted against *Salmonella enterica* (ATCC 10708), *Acinetobacter baumannii* (ATCC 19606), *Staphylococcus aureus* – MRSA (ATCC 33592), *Escherichia coli* O157:H7 (ATCC 43895), *Listeria monocytogenes* (ATCC 49594), and *Enterococcus faecium* VRE (ATCC 51559). Three lots (Lot Nos. J031891, J051291, and 060991) of the product, AdvaCare 120, were tested using Ecolab SOP Method MS042-14: "Antimicrobial Laundry Additives" (copy provided). The laboratory report referenced Petrocci and Clarke's "Proposed Test Method for Antimicrobial Laundry Additives." Each of the product lots tested was at least 60 days old at the time of testing. Use solutions of the product were prepared by adding approximately 1.2 g of the product and 2998.8 mL of 500 ppm AOAC synthetic hard water (titrated at 490-510 ppm; use solution at or below 61.66 ppm peroxyacetic acid/ 44.04 ppm hydrogen peroxide; a 1:2500 dilution). Cultures of the challenge microorganisms were prepared in accordance with Ecolab SOP Method MS042-14 (which is similar to culture preparation described in Petrocci and Clarke's method). Numerous sets of dry, sterile jars were filled with 75 mL of the prepared use solution and equilibrated to 140±2°F. The carriers for this test were prepared by boiling poly-cotton blend or 100% cotton cloth (thread count not specified) in a solution of 300.00 grams of sodium carbonate, 1.50 grams of Triton X-100, and 3 L of Milli-Q water. The fabric then was rinsed in boiling water and then rinsed in cold water. After drying for a minimum of 24 hours at ambient temperature, the fabric was cut into 2 inch wide strips weighing 15±1 grams. Each fabric strip was wrapped around a spindle. Swatches (1 inch by 1.5 inch) were also cut from the remaining fabric. Fabric carriers were sterilized by autoclave. Nine swatches per product lot per test organism were inoculated with 0.02 mL of the prepared organism culture, and dried until visibly dry but for no longer than 30 minutes at 35±2°C. After drying, the swatches were each inserted between the 6th and 7th lap of a wrapped spindle. Each spindle contained three dried, contaminated swatches. Three spindles were prepared for each test organism. The spindles were placed in the dry, sterile jars containing the use solution and subjected to a simulated tumble-wash at 35-60 RPM for 5 minutes at 140±2°F. Within 1 minute, the fabric swatches were transferred to 10 mL of DE Broth to neutralize. The fabric swatches were then vortex mixed well to extract fabric-bound microorganisms. Serial dilutions were prepared, and unspecified dilutions were plated on tryptone glucose extract agar. Also within 1 minute, 25 mL of 2% sodium thiosulfate was added to the wash water after the excess wash water was squeezed from the spindle using a sterile glove. The neutralized wash water was swirled, and a 1 mL aliquot was plated on tryptone glucose extract agar. All subcultures were incubated for 48±4 hours at 35±2°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for initial inoculum count, ambient wash water control count, test temperature wash water control count, ambient numbers

count, test temperature numbers count, purity, sterility, neutralization confirmation, and antibiotic resistance.

Note: Antibiotic resistance of *Staphylococcus aureus* – MRSA (ATCC 33592) was verified on a representative culture. An individual Mueller Hinton Agar was streaked with the prepared culture in three different directions. After streaking, antibiotic disks were added to the inoculated agar surface. The plate was incubated and, following incubation, the zone of inhibition was measured and documented. The measured zone of inhibition (i.e., 0 mm) confirmed antibiotic resistance of *Staphylococcus aureus* – MRSA (ATCC 33592) to methicillin. See pages 12 and 13 of the laboratory report.

Note: Antibiotic resistance of *Enterococcus faecium* VRE (ATCC 51559) was verified on a representative culture. An individual Mueller Hinton Agar was streaked with the prepared culture in three different directions. After streaking, antibiotic disks were added to the inoculated agar surface. The plate was incubated and, following incubation, the zone of inhibition was measured and documented. The measured zone of inhibition (i.e., 0 mm) confirmed antibiotic resistance of *Enterococcus faecium* VRE (ATCC 51559) to vancomycin. See pages 12 and 13 of the laboratory report.

Note: Adding a ca. 15-gram cloth strip to 75 mL of product use solution yields a 1:5 w/v ratio of simulated laundry to wash water, the appropriate ratio for institutional laundry additive testing.

Note: Protocol deviations/amendments reported in the study were reviewed and found to be acceptable.

V. RESULTS

MRID Number	Organism	No. Exhibiting Growth/ Total No. Tested			Carrier Counts (CFU/ Carrier)
		Lot No. J031891	Lot No. J051291	Lot Nos. 060991	
478975-01	*Klebsiella pneumoniae*				
	Fabric Carriers:	0 / 9	0 / 9	0 / 9	1.5×10^6
	Wash Water Plates:	0 / 3	0 / 3	0 / 3	6.4×10^4
	Staphylococcus aureus				
	Fabric Carriers:	0 / 9	0 / 9	0 / 9	1.5×10^7
	Wash Water Plates:	0 / 3	0 / 3	0 / 3	6.4×10^3
	Pseudomonas aeruginosa				
	Fabric Carriers:	0 / 9	0 / 9	0 / 9	3.7×10^6
	Wash Water Plates:	0 / 3	0 / 3	0 / 3	4.1×10^5
	Salmonella enterica				
	Fabric Carriers:	0 / 9	0 / 9	0 / 9	2.4×10^6
	Wash Water Plates:	0 / 3	0 / 3	0 / 3	1.7×10^5
	Acinetobacter baumannii				
	Fabric Carriers:	0 / 9	0 / 9	0 / 9	1.4×10^7
	Wash Water Plates:	0 / 3	0 / 3	0 / 3	9.4×10^4

MRID Number	Organism	No. Exhibiting Growth/ Total No. Tested			Carrier Counts (CFU/ Carrier)
		Lot No. J031891	Lot No. J051291	Lot Nos. 060991	
478975-02	*Staphylococcus aureus* – MRSA				
	Fabric Carriers:	0 / 9	0 / 9	0 / 9	9.2×10^6
	Wash Water Plates:	0 / 3	0 / 3	0 / 3	1.2×10^4
	Escherichia coli O157:H7				
	Fabric Carriers:	0 / 9	0 / 9	0 / 9	1.0×10^7
	Wash Water Plates:	0 / 3	0 / 3	0 / 3	1.0×10^6
	Listeria monocytogenes				
	Fabric Carriers:	0 / 9	0 / 9	0 / 9	4.5×10^6
	Wash Water Plates:	0 / 3	0 / 3	0 / 3	8.1×10^4
	Enterococcus faecium VRE				
	Fabric Carriers:	2 / 9	3 / 9	0 / 9	5.0×10^6
	Wash Water Plates:	0 / 3	0 / 3	0 / 3	1.5×10^5

VI. CONCLUSIONS

1. The submitted efficacy data **support** the use of the product, AdvaCare 120, as a laundry additive for disinfecting laundry during commercial-industrial-institutional laundry operations against the following microorganisms in the presence of 500 ppm hard water for a 5-minute contact time at 140±2°F at a 1:2500 dilution:

Klebsiella pneumoniae	MRID 478975-01
Staphylococcus aureus	MRID 478975-01
Pseudomonas aeruginosa	MRID 478975-01
Salmonella enterica	MRID 478975-02
Acinetobacter baumannii	MRID 478975-02
Staphylococcus aureus – MRSA	MRID 478975-02
Escherichia coli O157:H7	MRID 478975-02
Listeria monocytogenes	MRID 478975-02

Complete killing was observed in the subcultures of all fabric swatches and wash water tested against the required number of product lots. Each of the product lots tested was at least 60 days old at the time of testing. Neutralization confirmation testing of the fabric carriers showed positive growth of the microorganisms. Neutralization confirmation testing of the wash water met the acceptance criterion of growth within 20% of the other recovery numbers. Purity controls were reported as pure. Sterility controls did not show growth.

2. The submitted efficacy data (MRID 478975-02) **do not support** the use of the product, AdvaCare 120, as a laundry additive for disinfecting laundry during commercial-industrial-institutional laundry operations against *Enterococcus faecium* VRE in the presence of 500 ppm hard water for a 5-minute contact time at 140±2°F at a 1:2500 dilution. Growth was observed in 2 of 9 fabric swatch subcultures for one product lot, and 3 of 9 fabric swatch subcultures for a second product lot.

VII. LABEL

1. The proposed label claims that the product, AdvaCare 120 Sanitizer/Sour, is an effective laundry additive for disinfectant laundry against the following microorganisms in commercial-industrial-institutional laundry operations in the presence of 500 ppm hard water for a 5-minute contact time at 140±2°F when added at a rate of 4 ounces per 60 gallons of water (a 1:1919 dilution):

Klebsiella pneumoniae
Staphylococcus aureus
Pseudomonas aeruginosa
Salmonella enterica
Acinetobacter baumannii
Staphylococcus aureus – MRSA
Escherichia coli O157:H7
Listeria monocytogenes

These claims are acceptable as they **are supported** by the submitted data.

2. The following revisions to the proposed label must be made:

- Under the "Environmental Hazards" section of the proposed label, change "or **public** waters" to read "or **other** waters."

- Under the "To Disinfect and Bleach" section of the proposed label, change "**sanitize** a maximum" to read "**disinfect** a maximum." <u>This change should be made in two places</u>.

- Under the "Container Disposal" section of the proposed label, **identify disposal options for the container**.

- Change Acinetobacter baumanii" to "Acinetobacter baumannii"